AN ENVIRONMENTAL SENTINEL BIOMONITOR SYSTEM FOR DRINKING WATER PROTECTION

M.W. Widder*, D. Trader, L. Brennan, T.R. Shedd, T.P. Gargan II, and W.H. van der Schalie
U.S. Army Center for Environmental Health Research
Fort Detrick, MD 21702

N. Salazar
Agave Biosystems
Austin, TX 78708

Y.R. Vallejo
ANP Technologies, Inc.
Newark, DE 19711

ABSTRACT

Toxic industrial chemicals (TICs) are a potential threat to field drinking water supplies, but capabilities for rapid field testing of water are limited to relatively few chemicals. The Environmental Sentinel Biomonitor (ESB) system will significantly augment current detection methods by rapidly identifying toxicity from chemicals that currently cannot be rapidly detected in water samples in the field. The ESB system utilizes two toxicity sensors – a cell-based electrical impedance sensor and an enzyme-based acetylcholinesterase (AChE) inhibition sensor – to rapidly respond to a wide range of TICs. Future improvements to the ESB system under evaluation include the use of non-mammalian vertebrate cells to improve sensitivity and reduce logistical requirements and the development of an AChE inhibition sensor to provide more rapid detection with fewer steps and reagents.

1. INTRODUCTION

Field drinking water threats include toxic industrial chemicals (TICs), many of which are not produced or used in the US, as well as militarized chemical agents. Most drinking water used by troops today in Iraq and Afghanistan is produced using reverse osmosis-based systems that can efficiently remove most threat chemicals, but removal may be insufficient if chemicals are present in source waters at high concentrations or have been introduced after processing. Although preventive medicine personnel periodically test water supplies, field testing capabilities are limited to relatively few chemicals. More thorough (and costly) evaluations are done infrequently at remote laboratories, and test results are not available for days or weeks.

An alternative to traditional chemical-by-chemical analysis is to use biological systems to directly measure chemical-related toxicity. Biologically-based toxicity sensors can respond to a wide array of chemical contaminants, including chemical mixtures and novel or unsuspected materials. The ESB system will significantly augment current detection methods by providing a presumptive screening capability that can rapidly identify toxicity in water from chemicals that currently cannot be identified readily in theater.

This paper describes the response of ESB system toxicity sensors to TICs having varying modes of toxic action as well as to potential interfering chemicals that may be present in drinking water supplies. The current status of the ESB test platform and future improvements to the system are discussed.

2. METHODS

Toxicity sensors for the ESB system were selected based upon a formal decision analysis process that considered both Army needs and current technological capabilities (Kooistra et al., 2007). An evolutionary, spiral development course was recommended by an Integrated Product Team (IPT) of Army users, with the initial increment of the ESB system intended to support rear area and garrison drinking water evaluation needs. An initial downselection of 38 technologies led to comparative response testing of 12 toxicity sensors (van der Schalie et al., 2006). The best performing sensors were selected for further evaluation.

The toxicity sensors incorporated in the current prototype ESB system include an electric cell-

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substrate inhibition sensing (ECIS) device and a test for acetylcholinesterase (AChE) inhibition. The ECIS test (Agave Biosystems, Ithaca, NY) measures toxicant-induced changes in the electrical impedance of a cell monolayer over a one-hour period. Preliminary testing with bovine lung microvessel endothelial cells (BLMVEC) was conducted in open well fluidic chips. For definitive tests, cells are maintained in a novel two-channel fluidic chip (Figure 1); one channel is used as a control; the second channel receives water containing the toxicant. Each channel has four electrodes that are used to measure impedance of the overlying cells. Statistical differences in impedance curves between the control and toxicant-treated wells or channels are determined using an automated curve discrimination program incorporating functional data analysis techniques.

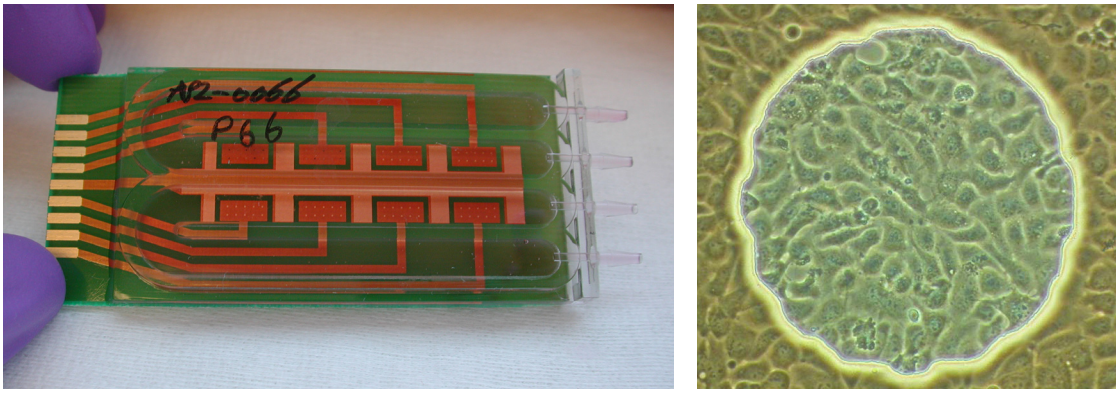

Figure 1. ECIS fluidic chip. A monolayer of BLMVEC cells covering an ECIS electrode is shown on the right.

An AChE inhibition test (Abraxis, Warminster, PA; Figure 2) was selected based on the results of an evaluation of a number of commercially-available kits (Buehler, 2007) using organophosphorus pesticides (fenamiphos, methamidophos, and methyl parathion) and carbamate pesticides (aldicarb and oxamyl) from the TIC test set. Inhibition of sample color development, indicative of AChE inhibition, was measured using a hand-held colorimeter (Hach, Inc., Loveland, CO) during the 75-minute test procedure. Inhibition of AChE activity by more than 20% from the control level was considered a positive effect.

ESB system toxicity sensors are intended to respond to toxicant concentrations exceeding 7- to 14-day Military Exposure Guideline (MEG) levels, assuming an individual water consumption rate of 15 L/day typical of arid environments (USACHPPM, 2004). The upper limit for toxicity sensor response was defined as the estimated human lethal concentration (HLC; TERA, 2006). Sensor responses to a set of TICs and potential interfering materials selected in consultation with the IPT were evaluated (Table 1). The TICs include diverse chemicals with

Figure 2. Abraxis test kit.

varying modes of toxic action; they are intended to represent the much larger set of potential threat chemicals. Potential interferences include chemicals commonly used for drinking water disinfection (chlorine and chloramine), byproducts of cyanobacteria blooms (geosmin and MIB) and plant decomposition (humic and fulvic acids) found in certain source waters. Hard water (water high in calcium and magnesium and associated anions) was included because of the potential sensitivity of some biological systems.

Table 1. Toxic Industrial and Potential Interfering Chemicals Used to Evaluate ESB System Toxicity Sensors

TICs	**Potential Interferences**
Acrylonitrile	Chloramine residual
Aldicarb	Chlorine residual
Ammonia	Geosmin
Copper sulfate	Humic/fulvic acid
Ethylene glycol	Methyl Isoborneol (MIB)
Fenamiphos	Water blank (hard water)
Mercuric chloride	
Methamidophos	
Methyl parathion	
Nicotine	
Oxamyl	
Paraquat dichloride	
Pentachlorophenol	
Phenol	
Sodium arsenite	
Sodium cyanide	
Sodium fluoroacetate	
Strychnine	
Thallium	
Toluene	

3. RESULTS AND DISCUSSION

3.1 Toxicity Sensor Responses to TICs and Interferences

Range-finding test results provided a preliminary indication of ESB system capabilities; definitive detection level testing is on schedule for completion in FY2008. Although security considerations do not allow toxicant-specific detection limits to be reported here, it can be stated that the ECIS test responded to 19 of the 20 TICs; a typical toxicant response is shown in Figure 3. Similar to Figure 3, most toxicants tested caused a rapid decrease in cellular impedance. As expected, the Abraxis test kit had greater sensitivity than ECIS to the tested TICs whose primary mode of toxic action is AChE inhibition: carbamate pesticides (aldicarb, oxamyl) and organophosphorus pesticides (fenamiphos, methamidophos, and methyl parathion). Overall, a combination of the ECIS and Abraxis test kits responded to 95% of the chemicals; 70% tested within the desired sensitivity range.

Of the potential interferences tested, the ECIS test was affected only by the disinfectants chlorine and chloramine; chlorine is typically added to field water immediately after production. These disinfectants can be neutralized by the addition of a mild reducing agent such as sodium bisulfite. In preliminary testing, the Abraxis test kit did not respond to high water hardness or humic and fulvic acids; tests with other interferences are planned.

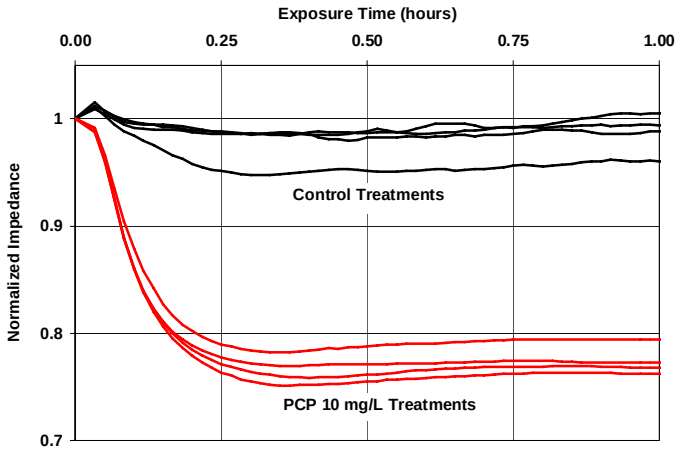

Figure 3. ECIS response to a 10 mg/L treatment of sodium pentachlorophenate (PCP). Black lines represent the impedances of cells overlaying four electrodes in the control channel of a fluidic chip; red lines are the impedances from electrodes in the PCP-treated channel.

3.2 ESB System Status

The prototype Cell Maintenance System (CMS) and ESB system platform are shown in Figure 4. Fluidic chips for ECIS testing are held in the CMS until needed for water testing. Each CMS holds 10 fluidic chips, and the ESB platform holds three CMS units. The CMS units provide temperature control and automatically supply fresh media to the fluidic chips as needed. To evaluate a water sample, the test sample is injected into one port of an ECIS chip, while a control sample (provided) is injected into the other port. The ESB system determines whether sample toxicity is indicated by cellular impedance changes. No user handling of the fluidic chips is required. The CMS unit can be returned for reloading with fresh ECIS chips. Abraxis test kit components and data analysis software are contained in the ESB system platform.

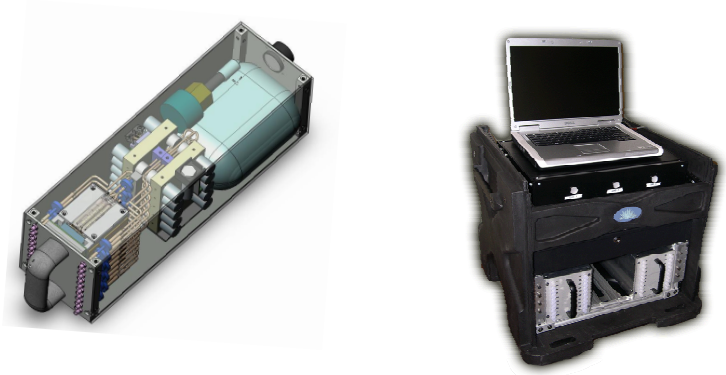

Figure 4. Prototype Cell Maintenance System (CMS, left) and the ESB platform (right). The ESB platform holds up to three CMS units. The Abraxis test kit is stored on the ESB platform.

The ESB platform requires external electrical power, which should not be a problem for its current intended use in rear areas and garrison facilities. Current shelf lives for required consumables are 10 to 12 months for the Abraxis test (refrigerated) and greater than 30 days for the ECIS fluidic chips;

maximum longevity of cells on ECIS fluidic chips is four months.

3.3 Future ESB System Improvements

Several promising research areas may result in substantial improvements in the toxicity sensors used in the prototype ESB system. For the ECIS test, it may be possible to replace the BLMVEC cells with non-mammalian vertebrate cells. BLMVEC cells, like other mammalian cells, require tightly-controlled environmental conditions and are difficult to store for long periods of time until they are needed for water sample testing. Two non-mammalian vertebrate cell lines are promising replacements for the BLMVEC cells because they have wider temperature tolerances, do not require carbon dioxide-enriched culture media, and show enhanced sensitivity to toxicants: amphibian melanophores and trout gill cells.

Certain lower vertebrates, such as amphibians and fish, are able to change their pattern of pigmentation. Cutaneous pigment cells, called chromatophores, can translocate their pigment granules and are the end effectors generating these 'chameleon'-like color changes in response to environmental conditions. When pigment granules are aggregated towards the nucleus, the cells appear transparent; when the pigment granules are dispersed, the cells appear colored. Changes in fish chromatophore transparency have been found to occur rapidly in response to many toxicants (Mojovic et al., 2004; Sharma et al., 2005; van der Schalie et al., 2006), but fish chromatophores are terminally-differentiated primary cells without the capacity for further cell division, so significant labor is required to obtain the chromatophores and there may be substantial variability in response between chromatophores obtained from different fish (Chaplen et al., 2002). Fortunately, one chromatophore cell type has been successfully immortalized: melanin-bearing cells (melanophores) from the amphibian *Xenopus laevis* (Daniolos et al., 1990; Potenza and Lerner, 1992). Pigment translocation in *X. laevis* melanophores occurs relatively rapidly (within 30 minutes) and can be monitored spectrophotometrically. Further, melanophores appear to be suitable for use in the ECIS system and preliminary data suggest that the melanophores can be maintained on fluidic chips for longer periods than the BLMVEC cells and with less frequent media replacement.

A rainbow trout (*Onchorynchus mykiss*) cell line derived from the gill tissues (RTgill-W1; Bols et al. 1994) has been found to work well in the ECIS system as well. The cells can be tested in simplified media, which enhances sensitivity to metals (Dayeh et al., 2005). As with the melanophores, it appears that the gill cells can be maintained on the fluidic chips longer than the BLMVEC cells with a much lower frequency of media replacement.

Although the Abraxis test kit provides excellent sensitivity to the organophosphorus and carbamate pesticides tested, test completion requires nine steps over a 75 minute period and requires reagents that must be refrigerated. With Phase II Small Business Innovation Research funding, ANP Technologies Inc. is developing an AChE inhibition assay that is projected to take less time and fewer steps while using reagents that have long shelf-lives without the need for refrigeration. In addition, the ANP device will use test tickets that are compatible with a handheld reader already used with analyte-specific immunoassay test tickets developed for Army field detection of biotoxins in water. The prototype AChE inhibition assay test tickets for use with the handheld reader are to be completed in December 2009.

CONCLUSIONS

The ESB system responds rapidly to a wide range of TICs with minimal interference issues, providing a capability not presently available for drinking water evaluation in deployed situations. Use in association with preventive medicine requirements for ensuring drinking water potability will provide an improved capability to rapidly detect chemical toxicity in field water. The initial prototype ESB system has been completed and is undergoing performance testing. Future ESB system improvements may include the use of robust cell types such as frog melanophores or trout gill cells as well as advanced enzyme-based testing to reduce sensor logistical requirements.

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